

PUPAL COLOR DIMORPHISM IN CALIFORNIA *BATTUS PHILENOR* (L.) (PAPILIONIDAE): MORTALITY FACTORS AND SELECTIVE ADVANTAGE¹

S. R. SIMS² AND A. M. SHAPIRO

Department of Entomology, University of California at Davis,
Davis, California 95616

ABSTRACT. Estimates of *Battus philenor* (L.) pupal mortality were made in central California. Summer mortality of first and second generation pupae from unspecified causes ranged from 9-20%. *Brachymeria ovata* (Say) (Hymenoptera: Chalcididae) attacked and killed *B. philenor* in the pupal stage. Rates of parasitism varied between populations but not between pupae on narrow twigs or broad tree trunk habitats.

A field experiment was conducted in a natural habitat of *B. philenor* to determine the selective advantage of pupal color dimorphism. Cryptic and non-cryptic pupae were affixed, in pairs, to narrow twigs in foliage or tree trunks and exposed to predators. Non-cryptic pupae in each pupation habitat suffered relatively more predation and lower survivorship. The extent of selective advantage conferred by cryptic coloration varied according to pupation substrate and season. Predation was greatest during the summer and on exposed tree trunks. The results indicate that *B. philenor* has greater survival on the pupation sites most frequently used in nature.

The pupae of *Battus philenor* (L.) are dimorphic, being either green or brown with rare intermediates. In the central Appalachian Mountains of Virginia, *philenor* pupates off the ground on broad exposed surfaces such as tree trunks and cliffs (Hazel & West, 1979). These pupae are almost always brown. California *philenor* also pupate off the ground but much more frequently on narrow twigs within green foliage (Sims & Shapiro, 1983). Two-thirds ($n = 1172$) of the California pupae found on narrow twigs (≤ 6 mm) are green. Pupae found on broad substrates such as grey concrete and brown tree trunks are predominantly (92%, $n = 283$) brown.

West and Hazel (1982) have shown higher survivorship of Virginia *philenor* pupae on the broad surfaces where they normally occur than on unutilized ground-level pupation sites in forest leaf litter. The difference in mortality between the two types of sites was attributed to the relative palatability of pupae to different predators. Birds hunting above ground level probably learned to avoid the distasteful pupae; whereas, the greater palatability of pupae to small mammals hunting at ground level may have led to the formation of search images.

The different pupation site distribution and color response of California *philenor* suggests that the selective mortality factors operating on apparent cryptic and non-cryptic pupae (background matching or

¹ Florida Agricultural Experiment Station Journal Series No. 5025.

² Current address: University of Florida AREC, 18905 S.W. 280th St., Homestead, Florida 33031.

contrasting respectively) are distinct from those of the eastern United States populations (Sims & Shapiro, 1983). This is not surprising considering differing factors in western areas such as the Mediterranean climate, riparian habitat of the foodplant (*Aristolochia californica* Torr.), evergreen nature of many associated dominant trees and shrubs, and probable differences in the species composition and predation pressure exerted by avian and other predators.

Since present "non-preference" for certain available pupation sites is possibly due to continuing selection against individuals with this behavior (see Clarke & Sheppard, 1972 for evidence for a genetic basis of pupation site choice in *Papilio polytes* L.), we tested the hypotheses that 1) greater survival of both cryptic and non-cryptic pupae occurs on "preferred" twigs, and 2) cryptic pupae have a selective advantage on twigs as well as on broad tree trunk environments.

Predation is not responsible for all mortality of *philenor* pupae. Pupae may be parasitized or die from undetermined causes. In this study we determined the percentage of pupae parasitized and compared rates of parasitization between pupae on narrow and wide substrates. We also estimated pupal death from undetermined causes over summer and winter.

METHODS AND MATERIALS

We studied parasitization and other mortality patterns of *philenor* pupae at Goethe Park, Sacramento Co., CA (Latitude 38°40'N) (GP) and Bidwell Park, Chico, Butte Co., CA (39°45'N) (BP). Parasite data was also collected on larvae from the Vaca Mountains (from Mix Canyon to Solano Lake, approximately 7 km SW Winters), Inner Coast Range, Solano Co., CA (38°25'N) (VM).

B. philenor pupae from GP were collected on twigs and tree trunks in an area adjacent to a 1 km stretch of the American River on seven sample dates from 25 January to 5 March 1974. The vegetation at GP is an oak-dominated riparian forest. The most common tree and shrub species are live oak (*Quercus agrifolia* Nee.), elderberry (*Sambucus mexicana* Presl.), redbud (*Cercis occidentalis* Torr. ex Gray), coffeeberry (*Rhamnus californica* Esch.), and the larval host, Dutchman's pipevine (*Aristolochia californica*).

BP is also a riparian habitat dominated by oak. Pupae here were sampled from either twigs of the host plant growing on concrete supports beneath a highway overpass or from the concrete itself.

Mortality in some *philenor* pupae is manifested by external discoloration and subsequent desiccation. This may result from disease, but the pathogen(s) remains undetermined. We estimated the magnitude of this type of mortality by collecting final instar larvae from VM and

BP and allowing them to pupate and remain within outdoor cages in a sheltered location in Davis, Yolo Co., CA.

We tested the selective advantage of cryptic pupal coloration on a 0.5 km stretch of south-facing slope in Mix Canyon (VM population) at an elevation of 100 m. Both *Aristolochia* and *philenor* are common in this area. Diapause pupae used in this test had been field-collected on 11 June 1976 at BP. They were affixed to tree trunks and narrow twigs using clear silicone rubber sealant. On 8–9 July 1976, 64 pairs of green and brown pupae were attached to opposite sides of individual *Q. agrifolia* tree trunks at heights of from 1 to 2 meters. An additional 40 pairs were attached to narrow (≤ 6 mm) twigs at similar heights in foliage of *Q. agrifolia*, *Heteromeles arbutifolia* M. Roem. (toyon), and *Umbellularia californica* (H. & A.) Nutt. (California bay). Pairs of pupae on twigs were separated by 30–40 cm. The location of pupae was marked by a stake at the base of the tree or shrub. Pupae were checked twice during the summer and once the following spring.

West and Hazel (1982) recognized several possible fates for individual pupae which are applicable here. A pupa may be: 1) alive and intact, 2) dead but intact, 3) attacked by a predator, remains visible, 4) gone and presumably predated, 5) eclosed. We assume that missing pupae are most likely predated and combine categories 3 and 4. The former position of pupae completely removed could generally be determined from the remaining traces of glue. The specific identities of probable predators were not determined in this study, although the visible remains of some predated pupae showed beak marks characteristic of birds.

RESULTS

The summer "disease" mortality, from June–September, of VM individuals pupating during June in 1975 and 1976 was 20.3% (total $n = 79$) and 15.2% ($n = 171$), respectively. BP had 8.6% "disease" mortality ($n = 558$) during the summer of 1976. The incidence of the "disease" may be higher in summer than during the following fall–winter and spring. For example, a sample of BP pupae collected on 25 January 1975 and monitored outdoors in Davis until emergence in March–April had only 4.6% ($n = 195$) dead pupae with these symptoms. This may be variable from year to year. In warm and wet years (such as 1981–1982) fungal attack may be a significant cause of death in overwintering pupae. In later winter, pupae so attacked show mildew in their spiracles and intersegmental membranes, but this is rarely recognizable by late spring.

During January–March 1974, 551 pupae were collected at GP. Most

pupae were found on stems and trunks of live oak, pipevine, redbud, and elderberry. Of 551 pupae, some appearing more than one year old, 427 (77.5%) were dead; 351 (82.5%) of the dead pupae had a 2–3 mm diameter circular opening in either the wing case or dorsum of thorax or abdomen suggesting a parasitoid emergence hole. Among dead individuals collected on narrow (≤ 6 mm) twigs, 79% ($n = 228$) had “parasite” holes while 86% ($n = 199$) of dead pupae on broad tree trunks (≥ 20 mm) had holes. These values are statistically similar ($\chi^2_1 = 3.54$, $P < 0.10$).

No parasitoids emerged the following spring from >1000 diapausing winter-collected pupae (GP 1974; BP 1975, 1976), nor were any parasitoids obtained from field-collected ova (>500 ova, VM 1973, 1974), 3rd instar larvae (>100 larvae, VM 1974), or final instar larvae (>400 larvae, VM 1974–1976; >200 larvae, BP 1975, 1976).

Of 755 pupae collected 24 June 1975 at BP, 3.4% produced adult *Brachymeria ovata* (Say) (Hymenoptera: Chalcididae). All parasitoid emergence was completed by 12 July. On 11 June 1976, a sample of 558 pupae had no parasitoids. Exit holes left by parasitoid adults from BP were identical to those seen in the GP pupae. *B. ovata* attacks and emerges from the pupal stage of many species of Lepidoptera (Harville, 1955; Peck, 1963).

The fate of green and brown pupae on narrow twigs and broad trunks is shown in Table 1. We compared the survivorship of cryptic vs. non-cryptic pupae between sites by combining the number of successful adult emergences over the summer with the number of surviving overwintering pupae. These comparisons are presented in Table 2. Most pupal mortality occurred during the summer, especially the first month following initiation of the experiment (Table 1). Green pupae on twigs and brown pupae on tree trunks suffered less predation than their alternate color forms but the differences were small (Table 2). The survivorship advantage of apparent cryptic coloration within sites in this study was largely due to greater adult emergence and lower non-predation mortality among the cryptic forms (Table 1). Our data suggests a lower survivorship of non-cryptic green pupae on tree trunks compared to brown pupae on twigs. Brown, and especially green pupae on tree trunks are quite conspicuous to the human observer and may be so to visually hunting predators. There was significantly reduced survivorship and increased predation on tree trunk pupae (combined green and brown) compared to twig sites (Table 2).

We did not identify any of the predators of *philenor*, but the few remaining predated pupae had beak mark damage characteristic of birds. Most pupae were removed completely or with only a bit of the abdomen and cremaster remaining.

TABLE 1. Survival of *B. philenor* pupae through the summer and winter of 1976-1977.

	Twigs						Tree trunks					
	Green pupae			Brown pupae			Green pupae			Brown pupae		
	Dead	Emerged	Predated	Alive	Dead	Emerged	Predated	Alive	Dead	Emerged	Predated	Alive
10 July	—	—	—	40	—	—	—	—	—	—	—	64
10 Aug.	5	15	3	17	9	12	6	13	5	35	11	23
15 Sept.	1	9	—	7	1	7	—	1	4	3	3	8
10 Mar.	—	—	—	7	—	—	—	—	—	—	—	6
Totals	6	24	3	—	10	19	6	—	14	9	38	—

TABLE 2. Survivorship (number of emerging adults during the year of the experiment + number of pupae alive the following spring) and predation of *B. philenor* pupae.

Pupal color and substrate	Survivors	Predated	χ^2 (1)	P
Green pupae on twigs	31	3	1.65	>0.10
Brown pupae on twigs	24	6		
Green pupae on tree trunks	12	38	3.40	<0.10
Brown pupae on tree trunks	24	35		
Total pupae on twigs	55	9	45.28	<0.01
Total pupae on tree trunks	36	73		

DISCUSSION

In this study we determined the magnitude of some *philenor* mortality factors in central California and the survivorship of cryptic and non-cryptic pupae on substrates which are most frequently used as natural pupation sites.

An estimated 9–20% of intact pupae die from unknown, possibly pathogenic, agents during the summer months. One sample indicated that winter mortality of pupae with disease symptoms is somewhat lower than summer, but this may vary from year to year.

The pupal-pupal parasitoid *Brachymeria ovata* attacks pupae in spring and summer. A single parasitoid is produced from each pupa, which is killed in the process. The parasitoids do not overwinter in diapause *philenor* pupae but probably overwinter as adults in a manner similar to other *Brachymeria* species (Clausen, 1940). Our determinations of percent parasitization may not accurately estimate the seasonal rate since we took only one sample each year per population. However, the rate of parasitization appeared low in the BP population, especially compared to the much higher estimate at GP. The determination of annual percent parasitization at GP is complicated by our inability to determine what proportion of the dead pupae in our samples represents individuals more than one year old. Thus, we have only an average estimate of parasitoid-caused annual mortality.

It is possible that synchronization between parasitoid activity and the months of greatest *philenor* pupation (May–June) influences parasitization rate. Individuals may not be “at risk” during their entire pupation period, since *Brachymeria* prefers to oviposit in newly-formed pupae (Clausen, 1940).

B. ovata has been observed to search randomly for host pupae in California habitats similar to those studied here (Harville, 1955). Random searching and host finding is reflected in the similar rates of parasitization of pupae on narrow and wide substrates. However, one

of us (AMS) has observed a parasitization rate in excess of 90% in each of four years at a site (Rossmoor Bar, not far from GP) where a great many pupae are formed in the open on whitewashed fence posts; no data are available for pupae from more natural substrates.

The primary pupal predators of California *philenor* remain undetermined. Several local nocturnal animals such as the opossum (*Didelphis virginiana*) and deer mouse (*Peromyscus* sp.) are possible candidates since they are often both arboreal and insectivorous (J. Harris, pers. comm.; Landry, 1970). Odor might be at least as important to these animals as visual cues. *B. philenor* adults are distasteful to some birds (Brower, 1958). Larvae are aposematically colored and it is likely that pupae possess alkaloids and aristolochic acid similar to those found in adults (Rothschild et al., 1970). We found some evidence for the unpalatability of pupae to birds since 5 of 82 pupae predated were clearly damaged by a bird's beak but were left uneaten. Similarly, West and Hazel (1982) observed 13 of 139 damaged but uneaten *philenor* pupae on tree trunks over a two year period in Virginia.

The significantly reduced predation pressure among narrow pupation substrates is correlated with the high percentage (33% of total) of brown pupae there. This suggests that brown pupae are as cryptic or otherwise protected as green pupae on twig sites and indicates the variability of the color-influencing stimuli of twigs. Tree trunks produce less ambiguous color-determining cues. Only 8% of the pupae on broad exposed sites are non-cryptic green (Sims and Shapiro, 1983).

Evidence is beginning to accumulate that a distinct selective advantage is accorded to individuals of dimorphic Lepidoptera species that choose an appropriate pupation site and have a cryptic pupal color response (Hidaka et al., 1959; Baker, 1970; Wiklund, 1975; West & Hazel, 1982). The choice of pupation site and ability of individuals to show a cryptic color response to the site's color and texture varies in different *philenor* populations (West & Hazel, 1979; Sims & Shapiro, 1983). We believe that this variability is related to a combination of the structure and seasonal phenology of the pupation-habitat coloration and the intensity of predation pressure. The latter is partly determined by predator species composition and density. In the deciduous forest of Virginia, most *philenor* pupae show a preference for rough exposed surfaces above ground level, are especially sensitive to the textural-optical qualities of these sites and, thus, pupate brown (West & Hazel, 1979; Hazel & West, 1979). In the partly evergreen habitat determined by central California's Mediterranean climate, narrow twigs in leafy areas are more frequently chosen, pupae are less sensitive to brown-producing stimuli, and more pupae are green (Sims & Shapiro, 1983). This study lends support to the hypothesis that pupation site preference

has evolved under differential selection by predators (West & Hazel, 1982). The highest summer and overwinter survival and least predation occurred on the narrow pupation sites most frequently used by *philenor* in nature.

ACKNOWLEDGMENTS

We thank S. O. Mattoon for assistance in the study of the BP population, F. Gould and D. A. West for comments on an earlier draft of the manuscript, and C. Satterwhite for manuscript preparation.

LITERATURE CITED

- BAKER, R. R. 1970. Bird predation as a selective pressure on the immature stages of the cabbage butterflies, *Pieris rapae* and *P. brassicae*. J. Zool. Lond. 162:43-59.
- BROWER, J. V. Z. 1958. Experimental studies of mimicry in some North American butterflies. Part II. *Battus philenor* and *Papilio troilus*, *P. polyxenes* and *P. glaucus*. Evolution 12:123-136.
- CLARKE, C. A. & P. M. SHEPPARD. 1972. Genetic and environmental factors influencing pupal color in the swallowtail butterflies *Battus philenor* (L.) and *Papilio polytes* L. J. Entomol. (A) 46:123-133.
- CLAUSEN, C. P. 1940. Entomophagous Insects. McGraw-Hill, NY. 688 pp.
- HARVILLE, J. P. 1955. Ecology and population dynamics of the California oak moth, *Phryganidia californica* Packard (Lepidoptera: Dioptidae). Microentomol. 20:83-166.
- HAZEL, W. N. & D. A. WEST. 1979. Environmental control of pupal colour in swallowtail butterflies (Lepidoptera: Papilioninae): *Battus philenor* (L.) and *Papilio polyxenes* Fabr. Ecol. Entomol. 4:393-400.
- HIDAKA, T., T. KIMURA & M. ONOSAKA. 1959. Experiments on the protective coloration of pupae of the swallowtail, *Papilio xuthus* L. Zool. Mag. 68:222-226. (In Japanese with English summary.)
- LANDRY, S. O. 1970. The Rodentia as omnivores. Quart. Rev. Biol. 45:351-372.
- PECK, O. 1963. A catalogue of the Nearctic Chalcidoidea (Insecta: Hymenoptera). Can. Entomol. Supp. 30. 1092 pp.
- ROTHSCHILD, M., T. REICHSTEIN, J. VON EUW, R. APLIN & R. R. M. HARMAN. 1970. Toxic Lepidoptera. Toxicon 8:293-299.
- SIMS, S. R. & A. M. SHAPIRO. 1983. Pupal color dimorphism in California *Battus philenor* (L.): Pupation sites, environmental control, and diapause linkage. Ecol. Entomol. 8:95-104.
- WEST, D. A. & W. N. HAZEL. 1979. Natural pupation sites of swallowtail butterflies (Lepidoptera: Papilioninae): *Papilio polyxenes* Fabr., *P. glaucus* L. and *Battus philenor* (L.). Ecol. Entomol. 4:387-392.
- . 1982. An experimental test of natural selection for pupation site in swallowtail butterflies. Evolution 36:152-159.
- WIKLUND, C. 1975. Pupal colour polymorphism and the survival in the field of cryptic versus non-cryptic pupae in *Papilio machaon* L. J. Roy. Soc. Lond. 127:73-84.